Dean JR (1998) Solid phase microextraction. In: *Extraction Methods for Environmental Analysis*, ch. 5. New York: John Wiley.

Djozan DJ and Assadi J (1997) A new porous-layer activated-charcoal-coated fused silica fiber: application for determination of BTEX compounds in water samples using headspace solid-phase microextraction and capillary gas chromatography. *Chromatographia* 45: 183–189.

Eisart R and Levsen K (1996) Solid-phase microextraction coupled to gas chromatography: new method for the analysis of organics in water. *Journal of Chromatography A* 733: 143–157.

Eisart R and Pawliszyn J (1997) Design of automated solid-phase microextraction for trace analysis of organic compounds in aqueous samples. *Journal of Chromatography A* 776: 293–303.

Hageman KJ, Mazeas L, Grabanski CB *et al*. Coupled subcritical water extraction with solid phase microextraction for determining semivolatile organics in environmental solids. *Analytical Chemistry* 68: 3892–3898.

Jia C, Luo Y and Pawliszyn J (1998) Solid phase microextraction combined with HPLC for determination of metal ions using crown ether as selective extracting reagent. *Journal of Microcolumn Separations* 10: 167–173.

Johansen SS and Pawliszyn J (1996) Trace analysis of hetero aromatic compounds (NSO) in water and polluted groundwater by solid-phase microextraction (SPME). *Journal of High Resolution Chromatography* 19: 627–632.

Moens L, De Smaele T, Dams R *et al*. (1997) Sensitive, simultaneous determination of organomercury, -lead and -tin compounds with headspace solid phase microextraction capillary gas chromatography combined with inductively coupled plasma mass spectrometry. *Analytical Chemistry* 69: 1604–1611.

Pawliszyn J (1997) *Solid Phase Microextraction*: *Theory and Practice*. New York: Wiley-VCH.

Sarrión MN, Santos FJ and Galceran MT (1998) Strategies for the analysis of chlorobenzenes in soils using solid-phase microextraction coupled with gas chromatography-ion trap mass spectrometry. *Journal of Chromatography A* 819: 197–209.

Yang Y, Miller DJ and Hawthorne SB (1998) Solid-phase microextraction of polychlorinated biphenyls. *Journal of Chromatography A* 800: 257–266.

Food Technology Applications

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Introduction

The chemicals responsible for off-flavours, malodours and taints in foods and beverages can originate from incidental contamination from environmental (outside) sources (e.g. air, water, packaging material, a contaminated ingredient) and from chemical reactions occurring within the food material itself (e.g. lipid oxidation, enzymatic action, microbial metabolic reactions). In addition, imbalance off-flavours can occur when certain ingredient components that are normally present and often essential to the product are present in abnormally high or low concentrations.

When significant off-flavour problems occur, one of the first priorities of the food chemist is to identify any volatile or semivolatile organic chemicals that may be responsible. Once the identity of the off-flavour chemical(s) has been established, it is possible to speculate on its mechanism of formation and then decide on what corrective actions to implement to eliminate recurrence of the problem in the future.

Analytical Strategy for Studying Off-Flavours

The following steps are commonly used when trying to determine which chemicals in a particular food or beverage sample are the most important contributors to off-flavours:

- Extraction of volatiles/semivolatiles. The chemicals responsible for the food taint must be extracted and usually concentrated from the food matrix. This sample preparation step is critical to success. To isolate and evaluate potential chemical components that are responsible for the food taint, analytes must be separated from interfering chemicals in the food matrix.
- Injection into the gas chromatograph (GC: with or without cryofocusing).
- Separation of extracted volatiles on a GC capillary column with a suitable liquid phase. It is not uncommon to miss important polar compounds because the chemicals do not chromatograph well on nonpolar phases. Often the extraction technique is blamed, but the problem could simply be that an inappropriate analytical capillary column was used for the separation. One example is not detecting volatile fatty acids because separation was attempted on a nonpolar column.

- Determination of peak odour by olfactometry. It is often advantageous to sniff peaks as they elute from the GC column. The odour characteristics and intensities of the eluting peaks can help the analyst determine if the chemical is a likely contributor to the malodour or off-flavour. A variety of olfactometry detectors are commercially available; olfactometry detectors with heated transfer lines are highly recommended.
- Determination of which volatiles/semivolatiles are the most potent contributors to the product's odour. Gas chromatography–olfactometry (GCO) analysis has evolved over time to include dilution techniques (Aroma Extraction Dilution Analysis, AEDA and CharmAnalysis), cross-modal matching (Osme) and maximum perceived intensity. Of these three GCO modifications, extract dilution techniques and cross-modal matching have become the most common techniques used in analytical work on food flavours. Further discussion of the various GCO techniques is beyond the scope of this article.

Perhaps the most critical and challenging step in the process of characterizing the flavour of foods is the sample preparation technique used to isolate/concentrate the flavour compounds from the food matrix. Since it is not uncommon for the chemicals responsible for food malodours to be present at p.p.b. and even p.p.t. levels, the extraction technique must collect as many molecules of off-flavour chemicals as possible for GCO analysis. If the goal is to identify the chemicals responsible for an off-flavour, the sample preparation method selected should extract a representative profile of as many organic volatiles/semivolatiles from the sample as possible. On the other hand, it is also important that the extraction technique does not introduce or create volatiles that are not in the food product. For example, sample preparation techniques that involve heating the sample (e.g. steam distillation) can generate artifact peaks in sample chromatograms, and these odiferous artifacts may be misinterpreted as the cause of the malodour/off-flavour problem.

This article will discuss why solid-phase microextraction (SPME) is such an excellent extraction/concentration technique for the study of food off-flavours and taints.

Advantages of SPME as an Extraction Technique

Chemicals responsible for off-flavours can be polar, semipolar and nonpolar and cover a wide range of functional groups, boiling points and molecular

Table 1 General comparison of common analyte extraction techniques for studying food aromas (actual parameters vary depending on sample matrix, analyte, type of GC detector)

	Sample matrix	*Sample size (g)*	*Detection limit*	*Range of volatiles analysed*				*Sample automation*	*Sample prep time (min)*
				Gases	*Volatile*	*Semivolatile*	*Nonvolatile*		
Static headspace (SH)	G/L/S	0.1–10	p.p.m.					Yes	5–30
Dynamic headspace (DH)/Tenax	L/S	1–1000	p.p.b.–p.p.t.					Yes	10–30
Solid-phase microextraction (SPME)	G/L/S	0.1–10	p.p.b.–p.p.t.					Yes	5–60
Solvent extraction (SE)	L/S	0.1–10	p.p.b.					No	> 30
Supercritical fluid extraction (SFE)	S[a]	0.1–10	p.p.b.					Yes	10–60
Direct thermal desorption (DTD)	S	0.001–0.10	p.p.b.					No	5–10

G, Gas; L, liquid; S, solid; p.p.t., parts per trillion; p.p.b., parts per billion; p.p.m., parts per million.
[a]Capable of analysing liquids, but usually requires binding of liquid portion of sample with an inert matrix material.

weights. As a result, no one analytical extraction/sample preparation method works in all cases. It is not uncommon that multiple sample preparation methods are required to identify the chemicals responsible for off-flavours and malodours in a particular sample.

Each sample preparation technique has advantages and disadvantages. The choice of a suitable sample preparation technique depends on several factors, including number of samples to be tested, how quickly results are needed, type of sample (matrix effects), the nature of the analytes of interest (i.e. functional group, molecular weight, boiling point, thermal stability, etc.), desired detection limits and required accuracy.

Table 1 compares a few popular extraction techniques used prior to GC analysis. Considering the wide range of sample sizes that can be analysed by SPME, the low detection limits, the wide range of analyte boiling points that can be analysed, the fact that SPME can be automated and the short sample preparation time, it is no surprise that SPME is rapidly growing in popularity. The low cost of SPME equipment is also an advantage.

One often overlooked benefit of SPME is its high precision and accuracy compared to other GC sampling techniques. Studies comparing the precision and accuracy of SPME to other GC sampling techniques show that analytical results based on SPME extraction are often more precise and accurate than results based on other sample preparation techniques.

Several polar and nonpolar fibres with varying affinities for specific classes of compounds are now available. As a result, SPME fibre type can be selected in order to optimize results for a particular analyte class. Compounds that interfere with the

Table 2 SPME fibre selection guide

Analyte class	*Fibre type*	*Linear range*
Acids (C_2–C_8)	Carboxen-PDMS	10 p.p.b.–1 p.p.m.
Acids (C_2–C_{15})	CW-DVB	50 p.p.b.–50 p.p.m.
Alcohols (C_1–C_8)	Carboxen-PDMS	10 p.p.b.–1 p.p.m.
Alcohols (C_1–C_{18})	CW-DVB	50 p.p.b.–75 p.p.m.
	Polyacrylate	100 p.p.b.–100 p.p.m.
Aldehydes (C_2–C_8)	Carboxen-PDMS	1 p.p.b.–500 p.p.b.
Aldehydes (C_3–C_{14})	100 μm PDMS	50 p.p.b.–50 p.p.m.
Amines	PDMS-DVB	50 p.p.b.–50 p.p.m.
Amphetamines	100 μm PDMS	100 p.p.b.–100 p.p.m.
	PDMS-DVB	50 p.p.b.–50 p.p.m.
Aromatic amines	PDMS-DVB	5 p.p.b.–1 p.p.m.
Barbiturates	PDMS-DVB	500 p.p.b.–100 p.p.m.
Benzidines	CW-DVB	5 p.p.b.–500 p.p.b.
Benzodiazepines	PDMS-DVB	100 p.p.b.–50 p.p.m.
Esters (C_3–C_{15})	100 μm PDMS	5 p.p.b.–10 p.p.m.
Esters (C_6–C_{18})	30 μm PDMS	5 p.p.b.–1 p.p.m.
Esters (C_{12}–C_{30})	7 μm PDMS	5 p.p.b.–1 p.p.m.
Ethers (C_4–C_{12})	Carboxen-PDMS	1 p.p.b.–500 p.p.m.
Explosives (nitroaromatics)	PDMS-DVB	1 p.p.b.–1 p.p.m.
Hydrocarbons (C_2–C_{10})	Carboxen-PDMS	10 p.p.b.–10 p.p.m.
Hydrocarbons (C_5–C_{20})	100 μm PDMS	500 p.p.t.–1 p.p.m.
Hydrocarbons (C_{10}–C_{30})	30 μm PDMS	100 p.p.t.–500 p.p.b.
Hydrocarbons (C_{20}–C_{40+})	7 μm PDMS	5 p.p.b.–500 p.p.b.
Ketones (C_3–C_9)	Carboxen-PDMS	5 p.p.b.–1 p.p.m.
Ketones (C_5–C_{12})	100 μm PDMS	5 p.p.b.–10 p.p.m.
Nitrosamines	PDMS-DVB	1 p.p.b.–200 p.p.b.
Polyaromatic hydrocarbons	100 μm PDMS	500 p.p.t.–1 p.p.m.
	30 μm PDMS	100 p.p.t.–500 p.p.b.
	7 μm PDMS	500 p.p.t.–500 p.p.b.
Polychlorinated biphenyls	30 μm PDMS	50 p.p.t.–500 p.p.b.
Pesticides, chlorinated	100 μm PDMS	50 p.p.t.–500 p.p.b.
	30 μm PDMS	25 p.p.b.–500 p.p.b.
Pesticides, nitrogen	Polyacrylate	50 p.p.t.–500 p.p.b.
Pesticides, phosphorus	100 μm PDMS	100 p.p.t.–1 p.p.m.
	Polyacrylate	100 p.p.t.–500 p.p.b.
Phenols	Polyacrylate	5 p.p.b.–500 p.p.b.
Surfactants	CW-TPR	1 p.p.m.–100 p.p.m.
Sulfur gases	Carboxen-PDMS	10 p.p.b.–10 p.p.m.
Terpenes	100 μm PDMS	1 p.p.b.–10 p.p.m.
Volatile organic chemicals	Carboxen-PDMS	100 p.p.t.–500 p.p.b.
	100 μm PDMS	20 p.p.b.–50 p.p.m.
	30 μm PDMS	100 p.p.b.–50 p.p.m.

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chromatography when the food extract is analysed by GC can be eliminated or at least minimized. If lipid oxidation is being studied, for example, the analyst could choose a Carboxen-PDMS fibre to measure aldehydes in the 1–500 p.p.b. range. If concentrations of aldehydes above 500 p.p.b. are present, the Carboxen-PDMS fibre will become saturated and a 100 μm PDMS fibre would be a better choice. A SPME fibre selection guide is shown in **Table 2**.

For some applications, the portability of SPME is an important advantage. After analytes are adsorbed on an SPME fibre, they can be maintained on the fibre for an extended period of time by sealing the end of the fibre with a septum. This allows for convenient field sampling. Perfumers have used this technique, for example, to extract aroma chemicals from flowers in greenhouses, as well as the fragrant chemicals from exotic flowers found in the canopy of tropical rainforests. Another example is a food chemist who is trying to determine if a malodour in a particular food product is being absorbed by the product because it has been stored near odiferous foods (e.g. spices) or perhaps industrial solvents. The food chemist can extract volatiles from the air in a warehouse or walk-in cooler with SPME, transport the SPME device with the trapped volatiles to the laboratory for GC analysis, and see if the GC profile matches the profile of a problem sample.

Retention characteristics are highly dependent on the fibre used and the volatility of the adsorbed analytes. Studies have shown that even highly volatile compounds can be stored on Carboxen-PDMS fibres for 3 days at room temperature without loss. The pore dynamics of Carboxen 1006 make it a true adsorbent. Retention of volatiles on 100 μm PDMS fibres, however, is not nearly as good. Even when fibres are stored at $-4^{\circ}C$, only the least volatile analytes will be retained.

Specific Applications of SPME for Resolving Food Taints

The examples and case studies that follow illustrate the advantages of SPME as a sample preparation tool for the study of off-flavours and malodours in foods and beverages.

Light-Induced Off-Flavours in Milk: SPME vs. Headspace Analysis

Two types of light-induced oxidation reactions occur in milk and dairy products. Initially, a burnt, oxidized flavour develops and predominates for approximately 2–3 days. Dairy technologists refer to this off-flavour note as light-activated flavour (LAF). Degradation of sulfur-containing amino acids of the serum (whey) proteins is probably responsible for this reaction. The exact reaction products for LAF have not been clearly elucidated. Methional [(3-methylthio)propanal], however, has been implicated as a possible contributor. Understanding the true impact that methional has on LAF is difficult to determine because it is relatively unstable and breaks down into more stable components, including mercaptans, sulfides and disulfides. Recently, researchers have postulated an alternative mechanism for the formation of dimethyl disulfide by singlet oxygen oxidation of methionine.

In addition to the poorly understood LAF off-flavour, a second type of light-induced off-flavour occurs in milk and is attributed to lipid oxidation. This off-flavour, often characterized as metallic or cardboard-like, usually develops after 2 days and does not dissipate. Aldehydes (especially pentanal and hexanal) and, to a lesser degree, ketones (e.g. 1-hexen-3-one and 1-nonen-3-one), alcohols and hydrocarbons have been observed to form in milk as a result of light-induced lipid oxidation reactions. When milk is exposed to light, various carbonyl compounds form from the reaction of light and oxygen with unsaturated fatty acids in the milk fat triglycerides and other milk fat components. Autoxidation of unsaturated fatty acids involves a free radical reaction, forming fat hydroperoxides that degrade to various malodorous compounds (e.g. hexanal, the predominant lipid reaction by-product in light-exposed milk in the case of linoleic acid).

In one recent study to quantitate pentanal and hexanal in light-abused milk (skim milk and 2% fat milk), a comparison was made using two different sample preparation techniques: dynamic headspace (DH) with a Tenax trap and SPME with a Carboxen-PDMS fibre. Results, which are summarized in **Table 3**, show that standard calibrations with SPME were more linear for both analytes in both types of milk samples than with DH. (Calibration was based on the method of additions technique using an internal standard of 4-methyl-2-pentanone.) Furthermore, the SPME method had about the same detection limit as the DH method. To test the precision of each method, four replicates spiked with 2 ng mL^{-1} of each aldehyde were compared for both types of milk samples. When coefficients of variations were calculated for this study, SPME proved to be more precise than DH.

For these particular samples and these particular analytes, SPME consistently demonstrated better precision without a sacrifice in sensitivity. Furthermore, none of the problems with carryover, background or artifact peaks that sometimes occur with DH systems were observed with the SPME experiments. No carryover peaks were detected in milk samples, even

Table 3 Comparison of the principal analytical parameters for pentanal and hexanal analysed by DH/GC-MS and SPME/GC-MS

Compound	*Sample*	*Analytical technique*	*Detection limit (ng mL^{-1})*	*Repeatability of four replicates at 2 ng mL^{-1} (coefficient of variation, %)*	*Linear least-squares correlation coefficients*[a]
Pentanal	Skim	DH	0.1	8.0	0.966
		SPME	0.1	1.9	0.990
Hexanal	Skim	DH	0.3	21.1	0.910
		SPME	0.5	7.1	0.995
Pentanal	2% Milk	DH	0.3	7.6	0.996
		SPME	0.3	2.1	0.999
Hexanal	2% Milk	DH	0.8	8.3	0.982
		SPME	0.8	4.9	0.993

[a]For calibration curve of five standards ranging from 0.0 to 30.0 ng mL^{-1}.

when injecting the SPME fibre immediately after it was used to analyse a milk sample spiked with high levels (500 ng mL^{-1}) of each of the following aldehydes: butanal, isopentanal, pentanal, hexanal, heptanal and octanal.

Because so many different parameters need to be optimized when performing DH and SPME experiments, care must be taken when comparing SPME and DH for precision, accuracy and sensitivity, and it is probably an over-simplification to say that one method is better than another. None the less, this work shows that SPME is a viable extraction technique for measuring oxidation products in milk and dairy products.

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Highly Volatile Malodorous Chemicals

Highly volatile compounds can be responsible for off-flavours and malodours and can be difficult to trap and isolate. DH techniques with Tenax trapping often fail to trap and detect low molecular weight polar compounds. Static headspace works well for highly volatile chemicals but may not be sensitive enough for some applications.

SPME is an ideal extraction tool for highly volatile analytes. Consider, for example, the analysis of acetaldehyde in buttermilk. Acetaldehyde has a boiling point of 21°C.

Acetaldehyde in buttermilk The delicate flavour associated with high quality cultured buttermilk is contributed by several bacterial metabolites, including lactic acid, traces of acetic and formic acids, ethanol, diacetyl and carbon dioxide. Two different types of bacteria are used in buttermilk starter cultures: the acid-producing types (usually strains of *Streptococcus lactis* or *S. cremoris*) and the aroma bacteria (usually *Leuconostoc citravorum*). Diacetyl, the major flavour component of buttermilk, is produced by the fermentation of citric acid by the aroma-producing bacteria.

One common type of off-flavour in buttermilk is called the green flavour defect. It is caused by the loss of diacetyl (by conversion to acetyl methylcarbinol by diacetyl reductase enzyme in the culture bacteria) and an increase in acetaldehyde production. Measuring the acetaldehyde to diacetyl ratio is a good way to monitor this flavour defect.

As shown in **Figure 1**, SPME (e.g. Carboxen-PDMS) is an excellent way to extract acetaldehyde, diacetyl, acetic acid and other flavour-important metabolites from buttermilk. Even with SPME, however, it is necessary to use cryofocusing (typically at −100°C) after thermal desorption from the SPME fibre and prior to injection into the GC capillary column. With cryofocusing, sharp GC peaks are obtained for acetaldehyde; without cryofocusing, the acetaldehyde peak may not be detected at all.

1,3-Pentadiene from sorbate degradation Testing for 1,3-pentadiene in foods and beverages is another example of how SPME can be used to quantitate a highly volatile malodorous compound. Sorbic acid (2,4-hexadienoic acid) and its water-soluble potassium salt are commonly used as food preservatives to prevent yeast and mould growth. Foods in which sorbate has commercially useful antimicrobial activity include baked goods, cheeses and other dairy products, confectionery products, dried fruits, fish products, fruit juices, jellies (with artificial sweeteners), syrup, vegetables and wine.

One problem with potassium sorbate is that some moulds in the genus *Penicillium* can grow in the presence of up to (approximately) 1.2% potassium sorbate. Furthermore, some of these moulds have the ability to decarboxylate sorbic acid, producing 1,3-pentadiene, a highly volatile compound with an extremely strong hydrocarbon-like odour (typically kerosene-like).

As in the case of testing for acetaldehyde in buttermilk, using SPME with a Carboxen-PDMS fibre and

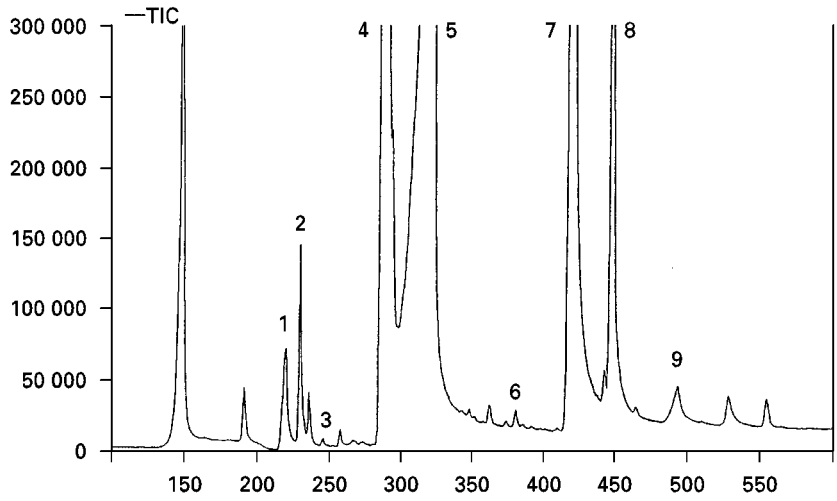

Figure 1 Volatiles in buttermilk by SPME (Carboxen-PDMS) extraction followed by GC-MS. Sample preparation: 2 mL of buttermilk, 7 μL of internal standard solution (54 p.p.m. 4-methyl-2-pentanone), and a small magnetic stirring bar were added to a 4 mL GC vial and sealed. Headspace volatiles were extracted by SPME for 20 min at 50°C. Peak identities: 1, acetaldehyde; 2, acetone; 3, dimethyl sulfide; 4, diacetyl; 5, acetic acid; 6, 2-pentanone; 7, ethyl acetate; 8, internal standard; 9, butyric acid.

cryofocusing prior to release into the analytical column works well for measuring 1,3-pentadiene in foods and beverages. A chromatogram showing 1,3-pentadiene in a ready-to-drink refrigerated tea product is shown in **Figure 2**. A consumer complained that this particular tea sample had a kerosene odour.

High Boiling Point Compounds with Musty Odours

While extremely volatile compounds can be challenging to extract and isolate, so too are high boiling point semivolatile chemicals. Sometimes it is necessary to use combinations of sample preparation techniques to extract and isolate sufficient quantities of this type of malodorous compound from foods to achieve meaningful analytical results.

Algae, fungi, bacteria and *Actinomycetes* are known to produce geosmin (GSM) and 2-methylisoborneol (MIB). These semivolatile, lipophilic compounds have a muddy, musty odour perceived as disagreeable to consumers. Both compounds are rapidly absorbed from water into the lipid tissue of fish and other aquatic organisms. When either compound is present in tissue at concentrations exceeding $0.7\ \mu g\ kg^{-1}$, they render fish unfit for retail sale.

Current methods for quantifying the concentrations of MIB and GSM in catfish include: purge-and-trap-solvent extraction (P&T-SE); microwave distillation–solvent extraction (MD-SE) and microwave distillation–solid phase extraction (MD-SPE). These methods are time-consuming, labour-intensive and require the use of small quantities of flammable and/or toxic solvents or expensive microwave equipment. A faster and less expensive method could find broad application in catfish flavour research, the catfish-processing industry and other aquaculture industries plagued by this problem.

Lloyd and Grimm, USDA research chemists, have developed a rapid and simple analytical procedure for quantitating low levels of GSM and MIB in catfish tissue. Their method combines microwave distillation (MD) with SPME. MD transfers lipophilic volatile analytes from the lipid-rich matrix of catfish tissue into an aqueous matrix, and SPME is then used to extract and concentrate the volatile organic compounds from the aqueous solution. The technique is a prime example of how combinations of two or more sample preparation techniques can be a potent strategy for resolving analytical problems that are inadequately addressed by a single sample preparation technique.

While SPME has been shown to be a sensitive, reproducible, quantitative sample preparation tool, the direct analysis of p.p.b. levels of GSM and MIB in fish tissue is not possible with SPME. Due to their lipophilic nature, MIB and GSM partition from fish tissue into the headspace in such low concentrations that direct SPME is ineffective. Combining MD with SPME yields a rapid, extremely sensitive technique for the analysis of thermally stable volatile and semivolatile compounds in complex matrixes. **Figure 3** is a schematic diagram of a typical MD-SPME apparatus for analysing MIB and GSM in fish tissue.

Mouldy/Musty Chemicals in Wine and Corks

Cork from *Quercus suber* has been used as a closure for wine bottles since the 17th century. Cork offers unique physical properties as a closure, including

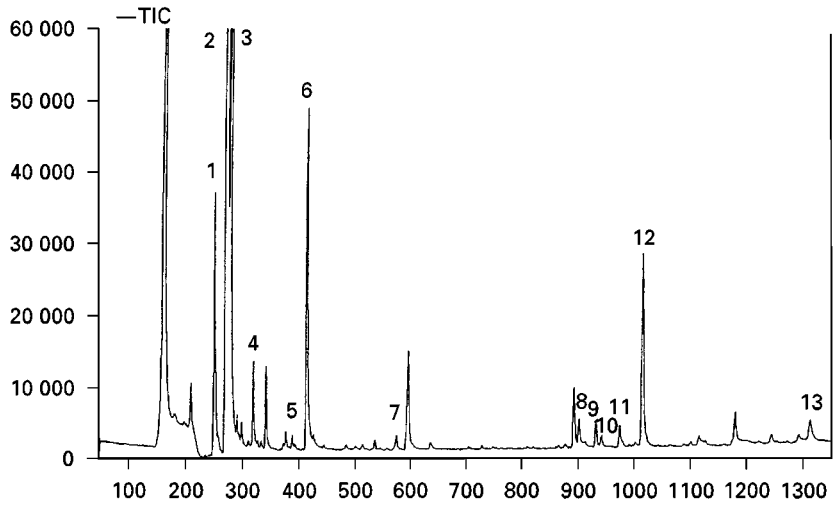

Figure 2 Volatiles in tea with a kerosene-like off-flavour by SPME (Carboxen-PDMS) extraction followed by GC-MS. Sample preparation: 2 mL of tea and a small magnetic stirring bar were added to a 4 mL GC vial and sealed. Headspace volatiles were extracted by SPME for 20 min at 50°C. Peak identities: 1, acetone; 2 and 3, 1,3-pentadiene isomers; 4, 2-butanone; 5, pentanal; 6, 2-pentanone; 7, hexanal; 8, 4-methyl-6-hepten-3-one; 9, 2,3-dehydro-1,8-cineole; 10, hexyl acetate; 11, 1,4-cineole; 12, 1,8-cineole; 13, α-terpineol.

long-lasting flexibility, hydrophobicity and gas impermeability. Over the last two decades, the incidence of mouldy and musty off-flavours in cork-sealed wines has increased significantly. 2,4,6-Trichloroanisole (TCA) has been identified as the primary chemical responsible for cork taint. The human olfactometry threshold for TCA is 4–10 ng L^{-1} in white wine and 50 ng L^{-1} in red wine. In the case of wine, a worldwide loss of roughly US$1 billion per year is attributed to cork taint.

The use of SPME fibres to extract TCA from the headspace over an agitated wine and moistened cork matrix is a short, inexpensive and solvent-free method to determine TCA. Due to the efficient adsorption properties of PDMS SPME fibres and the high sensitivity of GC-MS, the limit of detection of 2.9 ng L^{-1} TCA is low enough to detect problem wine and cork samples that exceed the olfactory threshold range in wine of 4–50 ng L^{-1}.

Immersion of the SPME fibre into the wine was found to give poorer sensitivity and can increase contamination of the injector system and shorten the lifetime of the SPME fibre and analytical GC column.

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Free Fatty Acids by Headspace and Immersion Techniques

Free fatty acids (FFAs), even at relatively low concentrations, are critical to both desirable and undesirable flavours in many types of food systems. Low levels of FFAs are difficult to detect in cheese and other food samples by dynamic or static headspace methods. SPME offers two alternative approaches to determine

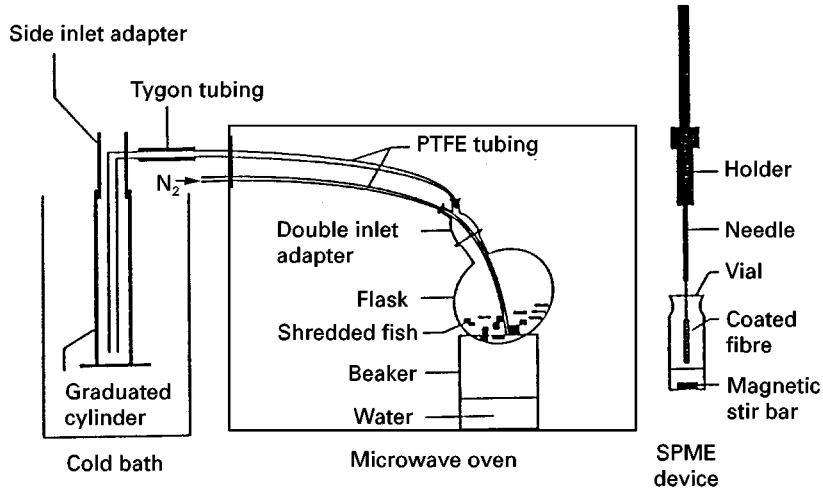

Figure 3 MD-SPME apparatus for analysing 2-methylisoborneol and geosmin in fish tissue.

Table 4 Linearity of responses for free fatty acids using immersion SPME (50 p.p.b.–25 p.p.m.)

Acid	*% RSD of response factor*
Acetic	140
Propionic	16.1
Isobutyric	14.4
Butyric	18.9
Isovaleric	12.1
Valeric	14.2
Hexanoic	9.1

Courtesy of Dr Robert Shirey, Supelco Inc., Bellefonte, PA.

these compounds in cheese: the solid sample can be warmed for headspace sampling, or the sample can be liquefied for sampling by immersion SPME. Shirey, Supelco's SPME applications chemist, investigated both approaches for monitoring FFAs in cheeses using varied extraction conditions.

The headspace SPME approach offered the greatest sensitivity for these analytes, but immersion of the fibre into the liquefied samples produced the widest range of linear responses. Under all conditions, acetic acid was particularly difficult to quantify (**Table 4**).

The following conditions were used for the analysis of Parmesan cheese for FFAs: sample: 100 mg cheese in 40 mL vial; SPME fibre: 65 μm Carbowax®/divinylbenzene StableFlex™; extraction method: headspace for 15 min at 65°C; desorption: 1 min at 250°C.

Sanitizer Contamination in Milk

The food and beverage industry is now less dependent on chlorine-based sanitizers for disinfecting processing equipment. Because application does not lead to toxic halogenated organic compounds, peroxyacetic acid (PAA)-based sanitizers are now widely used for disinfection in cleaning-in-place (CIP) systems in breweries and dairies. One problem with PAA-based sanitizers, however, is that even small amounts of PAA contamination can lead to severe off-flavours in milk. This problem can occur if sanitizers are not completely rinsed from processing lines prior to processing the next load of milk.

PAA, which can be quantitated in milk by HPLC after derivatization with methyl *p*-tolylsulfide, has a half-life in milk of approximately 20 min. As a result, PAA concentrations normally fall below threshold taste limits after only a few hours, even in milk contaminated with relatively large quantities of PAA. Once milk is contaminated with PAA, however, there is a significant off-flavour that fails to dissipate over time. The PAA-induced reactions that lead to this off-flavour defect are not well understood but probably involve oxidation of the milk proteins by PAA and/or hydrogen peroxide. To determine if an off-flavour in milk has occurred because of PAA contamination, one approach is to check acetic acid levels, since PAA degrades to water and acetic acid. Headspace SPME with a Carboxen-PDMS or a Carbowax-divinylbenzene StableFlex fibre is capable of detecting p.p.b. levels of acetic acid in milk.

One popular sanitizer used by some dairies is Matrixx™ (Ecolab, St Paul, MN). Matrixx has the following composition (approximate): 4.4% PAA, 6.9% hydrogen peroxide and 3.4% octanoic acid. **Figure 4** shows chromatograms of a control milk sample (no off-flavour) and a sample with a severe off-flavour that was suspected to be caused by contamination with Matrixx. Peaks for acetic and octanoic acids are indicators that the sample is contaminated with Matrixx sanitizer. The following conditions were used for the analysis: sample: 2 mL of low fat milk + 1 mL 0.1-N phosphoric acid + 1 g salt in a 9 mL vial; SPME fibre: 65 μm Carboxen-PDMS; extraction method: headspace (with stirring) for 12 min at 40°C; desorption: 2 min at 250°C. The analytical capillary column was FFAP™ (Free Fatty Acid Phase).

Off-Flavours from Packaging Materials

Ironically, packaging materials, which are designed to preserve the freshness and flavour of foods and beverages, can be directly responsible for causing off-flavour defects. Although plastic packaging material consists primarily of nonvolatile high molecular weight polymers, volatile low molecular weight compounds are often added to improve functional properties of the materials: plasticizers to improve flexibility, antioxidants to prevent oxidation of the plastic polymers and the food inside the packaging and UV blockers to prevent yellowing of polymeric material when it is exposed to light. Additional additives include polymerization accelerators, cross-linking agents, antistatic chemicals and lubricants.

Occasionally, packaging materials are not adequately cured before they are used. As a result, a small amount of solvent associated with the manufacturing of the packaging materials or from the inks and dyes used on packaging graphics remains and is absorbed by the food material inside the package.

Screening packaging material for undesirable residual solvents is a simple task with SPME. **Figure 5** shows volatiles extracted from the headspace of a closed, new (unused) cottage cheese carton (680 g fill weight). The lidstock is a linear low density polyethylene (Dow 2503 resin), and the container body is polypropylene (Montell copolymer). The volatiles were sampled simply by poking a pinhole through the top of the closed container and inserting an SPME fibre (Carboxen-PDMS) through the hole. A small magnetic stirring bar was placed inside the carton to

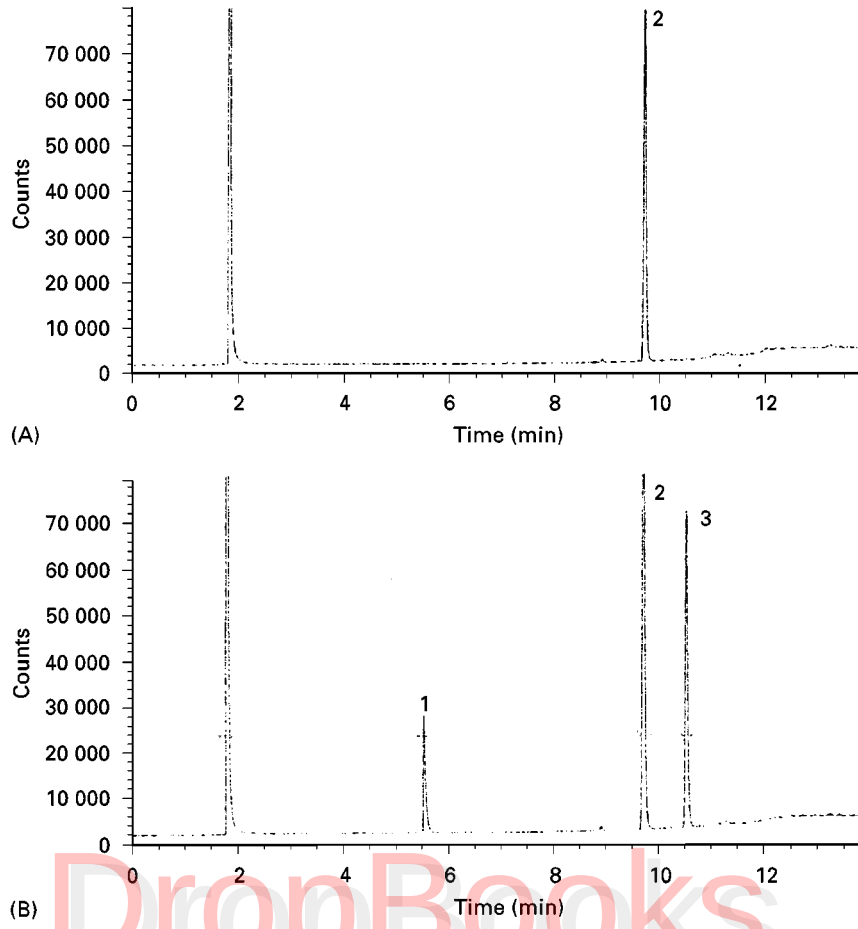

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Figure 4 (A) Low fat milk control and (B) complaint low fat milk with off-flavour. Peak identities are as follows: 1, acetic acid; 2, internal standard (2-ethylhexanoic acid); 3, octanoic acid. Complaint sample is contaminated with 0.11% Matrixx sanitizer. Concentration of octanic acid is 37 p.p.m. See text for details of method.

facilitate air movement over the fibre. The fibre was exposed to the atmosphere in the carton for 30 min at room temperature. A large number of volatiles was detected. Nearly all peaks detected were hydrocarbons of various chain lengths. However, a significant amount of trichloroethylene was also detected.

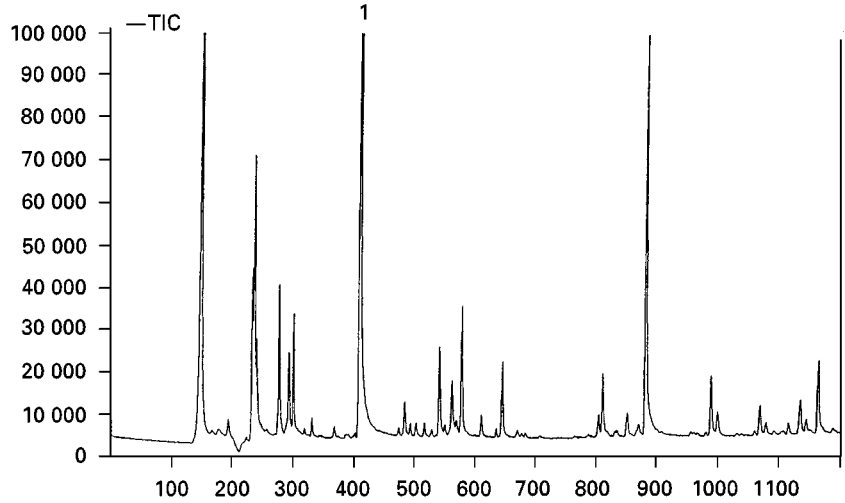

Figure 5 Volatiles extracted from the headspace of a closed, new (unused) cottage cheese carton (680 g fill weight) by SPME. Peak no. 1 is trichloroethylene; most of the other chromatographic peaks are alkanes. See text for details of method.

A few types of malodorous packaging solvents that have been found to cause off-flavours in foods include styrene, ethylstyrene, trimethylbenzene isomers and propyl acetate.

Pesticides in Wine

Not all food taints involve odiferous chemicals that contribute to off-flavours. Contamination of foods with pesticides is another type of food taint of critical concern. Wine is one type of beverage that can be contaminated with pesticides.

Procymidone fungicide Procymidone is a fungicide which is widely used against *Botrytis cinerea* on wine grapes. If improperly applied, undesirable residues at concentrations ranging from a few p.p.b. to several hundred p.p.b. can be found in wine after fermentation and even in old bottles because of its well-known persistence. The standard analytical sample preparation method for testing procymidone in wine is based on time-consuming liquid–liquid extraction or solid-phase extraction (SPE) using polymeric bonded silica cartridges.

Urruty and co-workers at the Université de Bordeaux (Périgueux, France) found that SPME (100 μm PDMS) results for procymidone in white and red wine correlated very well to ELISA test results. SPME was as fast as ELISA and offered slightly better precision.

Methyl isothiocyanate soil fumigant Another chemical of concern to wine makers is methyl isothiocyanate (MITC). It is used as a soil fumigant for nematodes, fungi and other diseases in vegetables and fruits. MITC is illegally employed as an antifermentative substance in wines. The addition of antifermentative agents in wines is controlled by EC and non-EC regulations. In particular, the Italian legal system does not allow the use of MITC in wines and requires the control of all exported wines. Solvent–solvent extraction is the traditional sample preparation method for measuring MITC in wines.

Grandini and Riguzzi (Bologna, Italy) compared SPME with the official Italian method. The SPME fibre used was Carbowax-divinylbenzene (65 μm). For SPME, headspace sampling of 5 mL of wine in a 10 mL vial was conducted for 30 min; 1.25 g of sodium chloride was added to the sample.

The lengthy standard sample preparation for MITC in wine was as follows: a 100 mL sample of wine was spiked with 100 μL 4-ethylpyridine (internal standard). The pH of the wine was adjusted to 7 with sodium hydroxide. The sample was then extracted three times with 15 mL of pentane. Anhydrous sodium sulfate was added to the solvent, which was then concentrated to 0.3 mL with a rotary evaporator at 40°C. No vacuum was applied, in order to minimize MITC loss.

SPME-GC with a nitrogen-phosphorus detector (NPD) gave a minimum detectable limit of 1 p.p.b. and a linear detector response in the 1–200 p.p.b. range. Although many methods use the NPD, including the official method, they are not able to obtain minimum detectable limits of less than 10 p.p.b. Compared to the official method, SPME offered the following advantages: low minimum detection limits, wide linearity range, short analysis time and low costs. Furthermore, sample pretreatment is eliminated and solvents are not used.

Quality Control (QC) Applications: SPME-MS-MVA as an Electronic Nose

The combination of SPME with GC and mass spectrometry–olfactometry detection is a potent tool for understanding the causes of food off-flavours, malodours and taints. However, the complexities involved in performing capillary GC testing, as well as the difficulties associated with the interpretation of results, require highly trained chemists. Furthermore, the technique is time-consuming and not amenable to the rapid product evaluation and decision-making that is often required in quality control situations. Even with assistance from peak recognition software that matches corresponding peaks in different chromatograms, the large number of GC peak data associated with flavour/off-flavour studies of food systems is time-consuming and prone to errors. As a result, SPME-GC-MS-OD is essentially a tool for research and development chemists and chromatographers.

Advantages of SPME-MS-MVA for QC Applications

There is, however, a relatively new SPME-based technique that has proved useful for food quality control applications. The technique has been referred to as SPME-MS-MVA (solid-phase microextraction–mass spectrometry–multivariate analysis). Essentially, the analytical system is an electronic nose (e-nose) in which a mass spectrometer replaces the typical chemical sensor array, and SPME replaces static or dynamic headspace sampling as the extraction technique to introduce volatiles/semivolatiles to the detector. The GC is used, with the only modification being the substitution of the typical 30 m coated capillary column with a 1 m uncoated fused silica column.

The speed, simplicity, sensitivity, portability and relatively low cost of SPME make it an ideal extraction technique for introducing volatiles and semivolatiles to the e-nose detector. With multiple manual SPME set-ups, it could be possible to analyse

one sample every 3 min using the same GC-MS system. Another advantage of using SPME as a way of introducing volatiles into the e-nose detector is that different fibres can be selected for different applications (see Table 2).

Using a mass spectrometer as a chemical sensor is advantageous because it is sensitive and robust, does not suffer from memory effects, and is not poisoned by low levels of moisture injected from SPME extractions. Furthermore, unlike typical commercial e-nose chemical sensors based on conducting polymers, metal oxides, surface acoustic wave (SAW) devices, quartz crystal microbalances (QCMs), or combinations of these devices, reliable easy-to-use benchtop MS detectors have been in routine use for decades and have a proven track record.

Another advantage of SPME-MS-MVA is that it can easily be converted to SPME-GC-MS simply by replacing the 1 m uncoated fused silica transfer line with an appropriate 30 m coated capillary GC column. Researchers can then perform more detailed traditional analyses, including identification and quantitation of specific odour-active GC peaks. This approach can be extremely helpful in determining what masses to monitor (as well as what masses to exclude) for specific e-nose application using MS as the chemical sensor.

Specific SPME-MS-MVA QC Applications

With SPME-MS-MVA, the ability to identify individual chemical components is lost. However, the trade-off is the gain in speed and simplicity of interpretation of results. The technique is rapid and generally gives comparative rather than quantitative information. It is ideally suited for quick quality assurance (QA)/QC screening.

SPME-MS-MVA generates mass intensity tables for each sample tested. The mass intensity data used to prepare the principal component analysis (PCA) scores plots in **Figures 6** and 7 were obtained in the following manner:

1. Sample volatiles were extracted using SPME (65 μm Carboxen-PDMS) and desorbed from the SPME fibre by the heated GC injection port (250°C) into a 1 m deactivated fused silica transfer line heated to 50°C.
2. Data acquisition (from m/z 50 to m/z 150) was discontinued after 2 min.
3. The masses of the single resulting chromatographic peak generated by the ion fragments from headspace volatiles of the sample were averaged from 8 to 80 s, while masses from 0 to 7 s and from 81 to 100 s were subtracted as background.

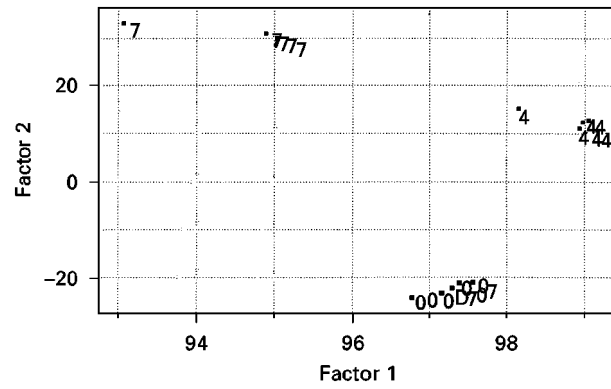

Figure 6 Principal component analysis scores plot of mass intensity data for control and light-abused soybean oils as determined by SPME-MS-MVA. Soybean oil: days of fluorescent light exposure (200 FC). 0, 0 days; 4, 4 days; 7, 7 days; D7, 7 days in the dark.

4. The resulting mass intensity list provided the data used for PCA.

Two QA/QC examples of SPME-MS-MVA are provided below.

Off-flavour development in soybean oil exposed to light Deodorized commercial soybean oil was exposed to fluorescent light for different time periods and analysed by SPME-MS-MVA. Prior to extraction, the soybean oil was placed in a 50 mL Nessler tube and exposed to 200 foot candles (FC) of fluorescent light. Four different types of samples were analysed: control soybean oil (fresh oil, normal taste, no light exposure); control oil exposed to light for 4 days; control oil exposed to light for 7 days; and a Nessler tube filled with control oil, wrapped in aluminium foil, and stored alongside the

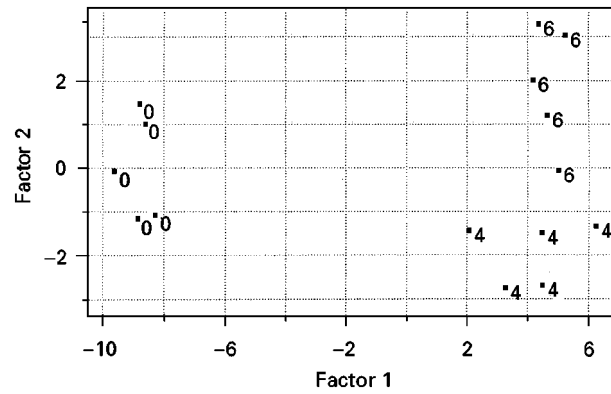

Figure 7 Principal component analysis scores plot of mass intensity data for fresh boiled beef and boiled beef refrigerated for 4 days and 6 days and then reheated. Results generated by SPME-MS-MVA technique. 0, 0 days (freshly boiled); 4, 4 days storage at 4°C; 6, 6 days storage at 4°C.

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light-exposed oils for 7 days. All Nessler tubes were sealed with Parafilm® and stored at 22°C. Six samples of each type were prepared and analysed, except for the 7-day-old sample stored in the dark (i.e. wrapped in foil); only three samples of this treatment were analysed.

SPME procedure 2 g soybean oil)was added to a 9 mL glass GC vial and capped with a polytetrafluoroethylene septum closure. Samples were heated to 45°C in a water bath and stirred vigorously with a small stirring bar while the SPME fibre was exposed to the headspace vapours in the vial for 12 min.

Results The PCA scores plot for this set of samples appears in Figure 6, which shows that SPME-MS-MVA is capable of grouping together samples of soybean oil that have been exposed to similar levels of light abuse.

Warmed-over flavour (WOF) in boiled beef A beef sample (500 g of chuck roast) was boiled for 60 min in a water bath. The internal temperature of the beef reached 92°C. Immediately after boiling, the hot meat was ground in a meat grinder, split into six separate samples and analysed by SPME-MS-MVA. After storage at 4°C for 4 days, the samples were reheated to 50°C in a convection oven for 30 min. Organoleptic evaluation of the samples showed that their flavour had changed from a typical beef flavour to an off-flavour characterized as tallowy, green and metallic. Samples were again refrigerated, stored for an additional 48 h, and re-analysed after warming to 50°C.

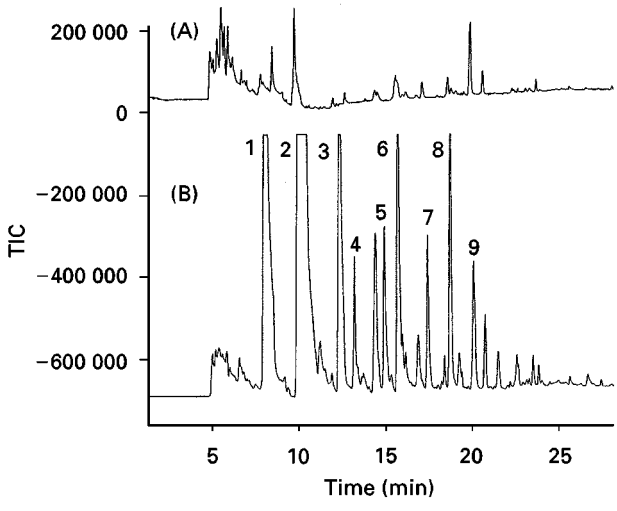

Figure 8 Development of warmed-over flavour in cooked beef. SPME-GC-MS chromatogram of boiled beef (A) at 0 days and (B) after 6 days of storage and then reheated to 50°C. Peak identities: 1, pentanal; 2, hexanal; 3, heptanal; 4, 2,4-nonadienal; 5, octanal; 6, 2,3-octanedione; 7, nonanal; 8, 1-octen-3-ol; 9, 2-heptenal.

Samples after 6 days of storage developed even stronger WOF notes.

SPME procedure 0.5 g boiled beef (ground) plus 2.5 mL water were added to a 9 mL glass GC vial. All other conditions were the same as the soybean oil SPME procedure given above.

Results The PCA scores plot for this set of samples appears in Figure 7. SPME-MS-MVA is capable of identifying groups of samples with similar levels of WOF.

To ensure that SPME was measuring volatiles that are known to contribute to WOF (e.g. aliphatic aldehydes, 2,4-nonadienal, etc.), a fresh boiled beef sample (0 days) and a 6-day sample were analysed by SPME-GC-MS. The resulting chromatograms, shown in **Figure 8**, prove that SPME is extracting compounds that have been identified as the source of WOF by other researchers. The chromatogram was generated using the identical method used for SPME-MS-MVA, with the exception that the 1 m transfer line was replaced with a 30 m FFAP capillary column.

Conclusion

As the numerous examples in this article illustrate, SPME is one of the most potent extraction, isolation and concentration techniques available for studying off-flavour chemicals in foods and beverages. Improvements in SPME technology will probably be made in the near future, making the technique even more useful to flavour chemists. Important recent developments in fibre technology include:

1. StableFlex™ fibres (which exhibit greater flexibility and increased strength compared to previous fibres);
2. a highly cross-linked PDMS fibre coating to minimize bleed and improve thermal stability;
3. coatings containing micro-adsorbent beads for retention and selectivity for many polar and volatile analytes;
4. dual-coated fibres that have the ability to efficiently extract low levels of both polar and nonpolar analytes in the same sample.

See also: **II/Chromatography: Gas:** Headspace Gas Chromatography. **Extraction:** Solid-Phase Microextraction. **III/Airborne Samples: Solid Phase Extraction. Fragrances: Gas Chromatography.**

Further Reading

Charalambous G (ed.) (1978) *Analysis of Foods and Beverages*. New York, NY: Academic Press.

Charalambous G (ed.) (1992) *Off-Flavors in Foods and Beverages*. Amsterdam: Elsevier Science.

Contis ET, Ho C-T, Mussinan CJ, Parliament TH, Shahidi F and Spanier AM (eds) (1998) *Food Flavors: Formation, Analysis and Packaging Influences*. Amsterdam: Elsevier Science.

Elmore JS, Mehmet AE and Muttram DS (1997) Comparison of dynamic headspace concentration of tenax with solid phase microextraction for the analysis of aroma volatiles. *Journal of Agriculture and Food Chemistry* 45: 2638–2641.

Heath HB and Reineccius G (eds) (1986) *Flavor Chemistry and Technology*. New York: Van Nostrand Reinhold.

Ho C-T and Manley CH (eds) (1993) *Flavor Measurement*. New York: Marcel Dekker.

Marsili RT (ed.) (1997) *Techniques for Analyzing Food Aromas*, pp. 237–289. New York: Marcel Dekker.

Marsili RT (1999) Comparison of solid phase microextraction and dynamic headspace method for the GC-MS analysis of light-induced lipid oxidation products in milk. *Journal of Chromatography Science* 37: 17–23.

Marsili RT (1999) SPME-MS-MVA as an electronic nose for the study of off-flavors in milk. *Journal of Agriculture and Food Chemistry* 47: 548–654.

Scheppers-Wercinski SA (ed.) (1999) *Solid Phase Microextraction: A Practical Approach*. New York: Marcel Dekker.

Overview

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Introduction

Solid phase microextraction (SPME) has been applied to a diverse range of analytes and sample types. The growth in the application of SPME, since its inception in 1990, can be seen in **Figure 1** (information from the *Science Citation Index*, February 1999). SPME is used as both a method of preconcentration and as a sampling device for (predominantly) chromatographic analysis. SPME has been used in conjunction with a range of other techniques, such as, ultraviolet and infrared spectroscopy, Raman spectroscopy and mass spectrometry, but it is its use in chromatographic analysis which is the focus of this article. SPME has most commonly been coupled to gas chromatography (GC), although some applications have coupled it to high-performance liquid chromatography (HPLC) (**Figure 2**). The following discussion will concentrate primarily on the use of SPME coupled with GC.

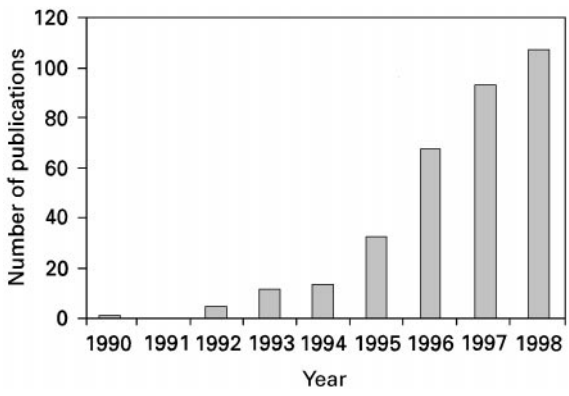

Figure 1 Frequency of SPME publications per year (information from the *Science Citation Index,* February 1999, Copyright International Scientific Communications, Inc.).

The SPME device consists of a fused silica fibre, coated with a stationary phase (**Table 1**) and mounted in a syringe-type holder (**Figure 3**). The SPME holder has two functions: to provide protection for the fibre and allow insertion into the hot environment of the GC injector using a needle. As samples and standards are normally introduced into a GC via a syringe the use of this device offers no additional complexity.

At rest the fused silica-coated fibre is retracted within the protective needle of the SPME holder. In operation however, the fibre is exposed to the analyte within its matrix (air, water, solid) for a predetermined amount of time. The active length of the fibre is typically 1 cm. Two common approaches for sample extraction are employed; direct and headspace (**Figure 4**). The first involves direct contact between the coated fibre and the sample matrix; in this way analytes within the sample are able to be transported to the fibre coating. This transportation can be achieved by several means. In the case of liquid (or solid samples that have been mixed with an aqueous solution, i.e. a slurry), transportation is achieved by agitation of the sample vial, agitation of the fibre, stirring or sonication of the sample solution. For gaseous samples, natural convection is usually sufficient. In the headspace mode, the process relies on the release of volatile compounds from the sample matrix. This may be achieved by heat, chemical modification or the inherent volatility of the analyte.

After sampling, the fibre is retracted within its holder for protection until inserted in the hot injector